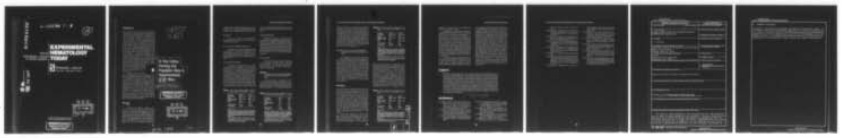
AD-A054 139 ARMED FORCES RADIOBIOLOGY RESEARCH INST BETHESDA MD F/G 6/16
IN VIVO COLONY FORMING UNIT POPULATION SIZES IN HYPERTRANSFUSED--ETC(U)
1977 K F MCCARTHY

UNCLASSIFIED NL

| OF |
AD
A054139



END
DATE
FILMED
6 -78
DDC

EXPERIMENTAL HEMATOLOGY TODAY

Edited by
SIEGMUND J. BAUM
G. DAVID LEDNEY

SPRINGER - VERLAG
NEW YORK HEIDELBERG BERLIN

(11) 1977

(12) 8p.

INTRODUCTION

As a result of a genetic defect expressed in the stroma of the tissues supporting hematopoiesis rather than in the hematopoietic cells themselves, mice of genotype *Sl/Sl*^{d} (Steel, Steel-Dickie mutant mice) suffer a chronic macrocytic anemia, and are extremely sensitive to ionizing radiation (11). Previously, it was hypothesized that the genetic defect disturbs erythropoiesis very early in the erythron, perhaps at the point of commitment of *in vivo* colony-forming units (CFU) to the erythrocytic cellular line of differentiation (8, 19). In an earlier study (9), this hypothesis was tested by measuring and comparing, in *Sl/Sl*^{d} mice and their congenic +/+ littermates, population sizes of high self-renewal potential and low self-renewal potential CFU. It was reasoned that a block in stem cell differentiation occurring early in the erythron would result in a deficiency of the latter but not of the former. However, our study did not bear this hypothesis out. Rather, it led to the unexpected observation that all the stem cell populations in *Sl/Sl*^{d} mice, with the exception of the splenic CFU population, were reduced in size.

9

However, anemia and other forms of hematopoietic stress are known to initiate substantial increases in extramedullary, but not medullary CFU population sizes (7, 10). and, because *Sl/Sl*^{d} mice suffer a chronic macrocytic anemia, it was reasoned that a comparison of the effects of *Sl* and + genes on CFU population sizes might be more meaningful if the comparison were undertaken not only on the same genetic background but also under similar physiologic conditions in which the blood RBC concentrations of +/+ and *Sl/Sl*^{d} mice are approximately the same. Therefore, in the present study, anemic *Sl/Sl*^{d} mice and normal +/+ mice were rendered polycythemic by hypertransfusion and the sizes of their CFU population determined.

(6) In Vivo Colony Forming Unit Population Sizes in Hypertransfused Sl/Sl^d Mice,

(10) Kenneth F./McCarthy

METHODS

MICE

WCB6F$_1$-*Sl/Sl*^{d}, B6D2F$_1$, C57BL/6J, and WC/Re mice were obtained from the Jackson Laboratory, Bar Harbor, Me. B6WCF$_1$ mice were raised at the Armed Forces Radiobiology Research Institute by mating C57BL/6J females with WC/Re males. The animals were maintained on a 6 AM to 6 PM (light-dark) cycle. Wayne Lab-Blox and acidified (pH 2.5) water were available *ad libitum*. All mice were acclimated to laboratory

conditions for 2 weeks. During this time they were certified free of lesions of murine pneumonia complex and of oropharyngeal *Pseudomonas* spr.

RADIATION

Mice were exposed to ^{60}Co wholebody gamma-radiation at a dose rate of 150 rad/min to a total dose of 950 rad.

IN VIVO COLONY FORMING ASSAY (CFU)

The CFU assay of Till and McCulloch (16) was performed as previously described (9). The following donor-recipient combinations were used: $B6D2F_1$ hematopoietic cells were transplanted into $B6D2F_1$ mice and $WCB6F_1$-*Sl/Sl*[d] into $B6WCF_1$ mice.

CFU SEEDING EFFICIENCY

The 2-hour seeding efficiencies *f* of CFU were determined according to the method of Siminovitch et al. (14). Briefly, in the case of femoral CFU, 6×10^6 marrow cells from a pool of one to four donor mice were injected into five intermediate recipients. Two hr later the mice were euthanized, their spleens removed, and 1/16 to 1/12 of a spleen was then injected into 10 secondary recipients. In the case of splenic CFU, $1-5 \times 10^7$ spleen cells from a pool of one to four donor mice were injected into five intermediate recipients. Two hours later 0.25–0.5 of a spleen was injected into four secondary recipients. The CFU content of the original cell suspensions was determined in primary recipients by the *in vivo* CFU assay.

HYPERTRANSFUSION

Blood for transfusion was collected from normal and heterozygous littermates from the orbital sinus into heparinized phosphate buffered saline and washed 3 times. One-half ml of washed packed red cells was then injected i.p. into each recipient daily on 3 successive days. Six days after the last injection, hematocrit (Hct.) values of the peripheral blood were determined. Mice having a Hct. of at least 55 were considered hypertransfused.

CALCULATIONS

CFU per 10^5 cells was calculated according to the formula: CFU/10^5 cells = observed colonies/10^5 cells $\times$ 1/*f*. CFU per organ was calculated according to the formula: CFU/organ = CFU/cell $\times$ cells/organ.

RESULTS

ORGAN CELLULARITY AND CFU NUMBERS IN NORMAL AND HYPERTRANSFUSED +/+ MICE

Presented in Table 1 and Table 2 are the number and colony-forming potential of nucleated cells from the spleen and femurs respectively of normal and hypertransfused male $B6D2F_1$ of genotype +/+. It was found, as has been

TABLE 1 Colony-Forming Ability of Spleen Cells from Normal and Hypertransfused $B6D2F_1$ – +/+ Mice

Hct. range	48–50	67–76
Colonies/10^5 cells	2.85 ± 0.73[a] [4][b]	3.60 ± 0.51 [3]
Nucleated cells/ spleen ($\times 10^{-7}$)	6.36 ± 0.85 [4]	8.35 ± 2.32 [3]
f (%)	14.8 ± 3.1 [4]	4.2 ± 1.2 [3]
CFU/10^5 cells[c]	19	86
CFU/spleen[d]	12,200	71,500

[a]Mean ± SE.

[b]Figures in brackets refer to number of separate determinations. Each determination consisted of a cell suspension prepared from three to four experimental mice being injected into seven to twelve recipient mice.

[c]Calculated from data for *f* and colonies/10^5 cells.

[d]Calculated from CFU/10^5 cells and average number of cells per donor spleen.

TABLE 2 Colony-Forming Ability of Marrow Cells from Normal and Hypertransfused $B6D2F_1$ – +/+ Mice

Hct. range	48–50	67–76
Colonies/10^5 cells	20 [1][a]	58 [1]
Nucleated cells/ femur ($\times 10^{-7}$)	1.07 [1]	1.13 [1]
f (%)	17.5 [1]	8.2 [1]
CFU/10^5 cells[b]	115	700
CFU/femur[c]	11,800	75,000

[a]Figures in brackets refer to number of separate determinations. Each determination consisted of a cell suspension prepared from four experimental mice being injected into twelve recipient mice.

[b]Calculated from data for *f* and colonies/10^5 cells.

[c]Calculated from CFU/10^5 cells and average number of cells per donor marrow.

reported by others (3, 13), that hypertransfusion increases the colony-forming potential of hematopoietic nucleated cells from both the marrow and spleen. Also, it was found that hypertransfusion decreases the CFU seeding efficiency 2–4-fold. Therefore, correcting for changes in both the spleen colony-forming potential and *f*, it was calculated that the femoral and splenic CFU population sizes of hypertransfused mice are approximately 4–6-fold larger on either a per cellular or per organ basis than are comparable CFU population sizes in normal mice.

ORGAN CELLULARITY AND CFU NUMBERS IN NORMAL AND HYPERTRANSFUSED *Sl/Sl^d* MICE

As compared to male mice of genotype +/+, hypertransfusion has exactly the opposite effect on the CFU populations of male *Sl/Sl^d* mice. Hypertransfusion (a) lowers the colony-forming potentials of the hematopoietic nucleated cells rather than increasing them; and (b) increases rather than decreases *f* (Tables 3 and 4). Taking these differences into consideration when calculating the CFU population sizes of hypertransfused *Sl/Sl^d* mice, as compared to anemic *Sl/Sl^d* mice, it was determined that hypertransfusion drastically reduces by about 50-fold the size of the splenic CFU population, and to a lesser extent—about 2-fold—the size of the marrow CFU population.

DISCUSSION

It was the tentative conclusion of a previous study (9) that the factors supporting a normal size splenic CFU population in *Sl/Sl^d* mice were predominantly long-range or systemic in nature, and were produced in response to the macrocytic anemia suffered by these mice (5). This hypothesis was tested in the present study by temporarily eliminating the anemia of *Sl/Sl^d* mice by hypertransfusion and measuring their CFU population sizes. It was found that hypertransfusion reduced the *Sl/Sl^d* splenic CFU population size 50-fold while reducing that of the marrow only 2-fold. In contrast, this same treatment increased both marrow and splenic CFU numbers of +/+ mice 6-fold.

When these findings are viewed in the light of the recent work of Bozzini et al. (1), they offer an insight into the puzzling fact that although erythropoiesis in *Sl/Sl^d* mice is erythropoietin-dependent (12), polycythemic

TABLE 3 Colony-Forming Ability of Spleen Cells from Normal and Hypertransfused WCB6F$_1$ – *Sl/Sl^d* Mice

Hct range	22–33	55–66
Colonies/10^5 cells	2.60 ± 0.82[a] [4][b]	0.27 ± 0.12 [4]
Nucleated cells/ spleen (× 10^{-8})	1.72 ± 0.21 [4]	1.34 ± 0.16 [4]
f (%)	4.4 [1]	14.7 ± 12.0 [2]
CFU/10^5 cells[c]	58.1	1.8
CFU/spleen[d]	102,000	2460

[a]Mean ± SE.

[b]Figures in brackets refer to number of separate determinations. Each determination consisted of a cell suspension prepared from one to two experimental mice being injected into seven to twelve recipient mice.

[c]Calculated from data for *f* and colonies/10^5 cells.

[d]Calculated from CFU/10^5 cells and average number of cells per donor spleen

Sl/Sl^d mice are refractory to exogenous erythropoietin (6). Bozzini and coworkers clearly demonstrated that the early reestablishment of erythropoiesis in polycythemic +/+ mice by exogenous erythropoietin is nearly *in toto* a splenic phenomenon. Therefore, given the stem cell-progenitor cell relationship of CFU to erythropoietin-responsive cells (ERC), the lack of a splenic erythropoietin-responsive compartment in hypertransfused Sl/Sl^d mice is consistent with the present finding of a nearly total absence of splenic CFU in these mice. As such, it might be concluded that the refractory character of hypertransfused *Sl/Sl^d* mice to exogenous eryth-

TABLE 4 Colony-Forming Ability of Marrow Cells from Normal and Hypertransfused WCB6F$_1$ – *Sl/Sl^d* Mice

Hct. range	22–33	55–64
Colonies/10^5 cells	15.00 ± 3.50[a] [2][b]	12.35 ± 1.35 [2]
Nucleated cells/ femur (× 10^{-7})	1.09 ± 0.16 [2]	1.51 ± 0.09 [2]
f (%)	7.3 [1]	15.0 [1]
CFU/10^5 cells	185	82
CFU/femur	20,200[c]	12,400

[a]Mean ± SE.

[b]Figures in brackets refer to number of separate determinations. Each determination consisted of a cell suspension prepared from one to two experimental mice being injected into seven to twelve recipient mice.

[c]Calculated from CFU/10^5 cells and average number of cells per donor femur.

ropoietin is a result, in part, of anomalous cell kinetics at the stem cell level.

In addition to a long-range mechanism regulating CFU proliferations, there is considerable evidence for a local one. For example, it is known that the selective depopulation of a hematopoietic cell maturation compartment, such as the erythron, results in the recruitment of CFU into cell cycle (3, 18). However, in the *Sl/Sl*^{d} mouse, suppression of the erythron by hypertransfusion does not appear to stimulate CFU proliferation. This might suggest that the stromal tissues of *Sl/Sl*^{d} mice are incapable of producing an effective local CFU proliferative factor in response to a depleted erythron. Indeed, similar observations on the absence of a local CFU proliferative mechanism in *Sl/Sl*^{d} mice have been reported by others (4, 15).

Given the concepts that (a) commitment of hematopoietic stem cells to the erythrocytic cellular line of differentiation is regulated by local stromal tissue, i.e., the hematopoietic inductive microenvironment (HIM), and (b) the *Sl* element is an integral part of this microenvironment (17), it might follow from the present work that the erythrocytic HIM can be described, in part, as a feedback loop between the erythron and the multipotent CFU compartment via the specialized stromal tissues supporting hematopoiesis. The mechanism would operate in such a fashion that CFU proliferation would be stimulated by a stromal tissue-CFU interaction in response to a depleted erythron. The exact nature of this stromal-supported CFU proliferation is, of course, not known. However, it could be speculated that this proliferative mechanism by itself or in unison with other factors generates and/or, amplifies a CFU subpopulation with a high capacity for erythroid differentiation. It is known that CFU of *Sl/Sl*^{d} origin have considerably less potential for establishing erythroid colonies in radiated recipient mice than do CFU of +/+ origin (17, 19) and, further, what erythropoiesis that does take place in *Sl/Sl*^{d} mice does so at erythropoietin concentrations characteristic of *in vitro* rather than *in vivo* systems (2, 6).

SUMMARY

The effect of hypertransfusion on the colony-forming unit (CFU) population size of normal and mutant *Sl/Sl*^{d} mice was determined. The main finding was that hypertransfusion reduced the splenic CFU population of *Sl/Sl*^{d} mice nearly 50-fold while increasing that of normal mice 6-fold. Hypertransfusion also reduced the marrow CFU population of *Sl/Sl*^{d} mice, but the reduction was only 2-fold. In normal mice, hypertransfusion resulted in a 6-fold increase in the marrow CFU population. Two tentative conclusions were drawn from the present study: (a) the refractoriness of the polycythemic *Sl/Sl*^{d} mice to exogenous erythropoietin is a result of anomalous stem cell kinetics characterizing the hypertransfused *Sl/Sl*^{d} mouse; and (b) the hematopoietic inductive microenvironment can be described, in part, as a feedback loop between the erythron and multipotent CFU compartment via the specialized stromal tissues which support hematopoiesis.

Sl/Sl superscripted

REFERENCES

(1) Bozzini, C. E., Martinez, M. A., Alvarez-Ugarte, C. A., Montangero, V., and Soriano, G. The importance of the spleen on proliferation of erythropoietin-responsive cells induced by erythropoietin. *Exp. Hematol.*, *2*:93, 1974.

(2) Gregory, C. J. Erythropoietin sensitivity as a differentiation marker in the hemopoietic system: Studies of three erythropoietic colony responses in culture. *J. Cell. Physiol.*, *89*:?89, 1976.

(3) Guzman, E., and Lajtha, L. G. Some comparisons of the kinetic properties of femoral and splenic haemopoietic stem cells. *Cell Tissue Kinet.*, *3*:91, 1970.

(4) Fried, W., Chamberlin, W., Knospe, W. H., Husseini, S., and Trobaugh, F. E., Jr. Studies of the defective haematopoietic microenvironment of *Sl/Sl*^{d} mice. *Br. J. Haematol.*, *24*:643, 1973.

(5) Harrison, D. E., Malath, V. G., and Silber, R. Elevated erythrocyte nucleoside deaminase levels in genetically anemic W/W^v and *Sl/Sl*^{d} mice. *Blood Cells*, *1*:605, 1975.

(6) Harrison, D. E., and Russell, E. S. The response of W/W^v and *Sl/Sl*^{d} anaemic mice to haemopoietic stimuli. *Br. J. Haematol.*, *22*:155, 1972.

(7) Hodgson, G. S., Bradley, T. R., and Telfer, P. A. Haemopoietic stem cells in experimental

haemolytic anemia. *Cell Tissue Kinet.*, *5*:283, 1972.

(8) McCarthy, K. F., and MacVittie, T. J. Erythrocytic committed hematopoietic stem cells in the peripheral blood of mice. *Acta Haematol.*, *53*:226, 1975.

(9) McCarthy, K. F., Ledney, G. D., and Mitchell, R. A deficiency of hematopoietic stem cells in steel mice. *Cell Tissue Kinet.*, in press, 1976.

(10) Rencricca, N. J., Rizzoli, V., Howard, D., Duffy, P., and Stohlman, F., Jr. Stem cell migration and proliferation during severe anemia. *Blood*, *36*:764, 1970.

(11) Russell, E. S., and Bernstein, S. E. Blood and blood formation. In Green, E. L., ed., *Biology of the Laboratory Mouse*. New York: McGraw-Hill, 1966, 2nd ed., p. 315.

(12) Schooley, J. C. as quoted in Harrison and Russell 1972.

(13) Schooley, J. C. Studies of stem cell kinetics utilizing the spleen colony technique and the erythropoietin sensitivity test. In Bond, U. P., and Sugahara, T., eds., *Comparative Cellular and Species Radiosensitivity*. Tokyo: Igaku Shoin Ltd., 1969.

(14) Siminovitch, L., McCulloch, E. A., and Till, J. E. The distribution of colony-forming cells among spleen colonies. *J. Cell. and Comp. Physiol.*, *62*:327, 1963.

(15) Sutherland, D. J. A., Till, J. E., and McCulloch, E. A. A kinetic study of the genetic control of hemopoietic progenitor cells assayed in culture and *in vitro*. *J. Cell. Physiol.*, *75*:267, 1970.

(16) Till, J. E., and McCulloch, E. A. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat. Res.*, *14*:213, 1961.

(17) Trentin, J. J. Determination of bone marrow stem cell differentiation by stromal hemopoietic inductive microenvironments (HIM). *Am. J. Pathol. 65*:621, 1971.

(18) Vassort, F., Winterholer, M., Frindel, E., and Tubiana, M. Kinetic parameters of bone marrow stem cells using *in vivo* suicide by tritiated thymidine or by hydroxyurea. *Blood*, *41*:789, 1973.

(19) Wolf, N. S. Dissecting the hematopoietic microenvironment. I. Stem cell lodgment and commitment, and the proliferation and differentiation of erythropoietic descendants in the *Sl/Sl^d* mouse. *Cell Tissue Kinet.*, *7*:89, 1974.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* IN VIVO COLONY FORMING UNIT POPULATION SIZES IN HYPERTRANSFUSED Sl/Sl^d MICE		5. TYPE OF REPORT & PERIOD COVERED 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) K. F. McCarthy		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Armed Forces Radiobiology Research Institute(AFRRI) Defense Nuclear Agency Bethesda, Maryland 20014		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Director Defense Nuclear Agency (DNA) Washington, D.C. 20305		12. REPORT DATE 13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS*(if different from Controlling Office)*		15. SECURITY CLASS. *(of this report)* UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

Published in Book Experimental Hematology Today

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The effect of hypertransfusion on the colony-forming unit (CFU) population size of normal and mutant Sl/Sl^d mice was determined. The main finding was that hypertransfusion reduced the splenic CFU population of Sl/Sl^d mice nearly 50-fold while increasing that of normal mice 6-fold. Hypertransfusion also reduced the marrow CFU population of Sl/Sl^d mice, but the reduction was only 2-fold. In normal mice, hypertransfusion resulted in a 6-fold increase in the marrow CFU

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

20. ABSTRACT (continued)

population. Two tentative conclusions were drawn from the present study: (a) the refractoriness of the polycythemic Sl/Sl^d mice to exogenous erythropoietin is a result of anomalous stem cell kinetics characterizing the hypertransfused Sl/Sl^d mouse; and (b) the hematopoietic inductive microenvironment can be described, in part, as a feedback loop between the erythron and multipotent CFU compartment via the specialized stromal tissues which support hematopoiesis.